DETERMINATION OF TOTAL NITROGEN,[1] NITRATE-NITROGEN, AND TOTAL NITROGEN NOT INCLUDING NITRATE-NITROGEN: FURTHER OBSERVATIONS ON A MODIFICATION OF THE OFFICIAL SALICYLIC-THIOSULPHATE METHOD[2]

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INTRODUCTION

The data here presented are in continuation of a series of investigations previously reported (Ranker, '25). These previous data were presented in support of a proposed modification of the official salicylic-thiosulphate method for the determination of total nitrogen including nitrate-nitrogen. The recommended procedure is as follows:

"Place the sample in an 800-cc. Kjeldahl flask; adjust to neutrality or make slightly alkaline; if water is present evaporate *just to* dryness on a water bath under vacuum. Add 35–40 cc. of salicylic acid mixture (1.0 gm. of salicylic acid to 30 cc. of concentrated nitrogen-free sulphuric acid); mix thoroughly and allow to stand for at least an hour with occasional shaking (if organic matter is present, stopper tightly with a rubber cork and allow to stand over night). Add 5 gms. of sodium thiosulphate and heat for 5 minutes with a low flame; cool; add 7–10 gms. of anhydrous sodium sulphate and a pinch of copper sulphate. Digest for an hour at the boiling point after the solution clears; just before the solution solidifies dilute to an estimated volume of 400 cc.; cool completely. Add a small piece of paraffin, 100 cc. of a saturated solution of sodium hydroxide, and a piece of mossy zinc; connect immediately to the distillation apparatus and distill 150–200 cc. over into standard acid during a period of 1 hour. Titrate the standard acid to neutrality with standard alkali and calculate the amount of nitrogen present."

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[1] By the unqualified term "total nitrogen" is meant the sum total of all forms of nitrogen present in the particular sample of material being analyzed.

[2] The second part of a series of investigations carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

In the interest of brevity this proposed method will be referred to as the "modified official method." Certain further applications of the modified official method seemed desirable, and at the same time such an extension would afford opportunity to test its accuracy more extensively. The work has progressed along several lines and certain phases of the investigation are being continued.

In the work previously referred to (Ranker, '25, table I) it was found that only 92.7 ± .90 per cent of the total nitrogen present was recovered by the modified official method, from samples containing "50 mgs. nitrate-nitrogen plus 0.5 gm. sucrose." In contrast 98.1 ± .64 and 99.5 ± .00 per cent of the total nitrogen were recovered by the comparison method used. Identical samples were used in all cases. From these data it was concluded (page 371) that "if sugar is present in abundance a slight loss of nitrate-nitrogen may occur, due to the reducing action of the sugar." It was concluded further that "this loss would be very slight in actual practice since the nitrate-nitrogen content of plants is small." The last conclusion is based on assumption only. In order to ascertain the true condition that would maintain "in actual practice" some analyses were made to determine the accuracy of the modified official method for samples of plant materials high in sugar and nitrate-nitrogen content. These data are given in table I.

Strowd ('20) concluded that "the determination of nitrates in plants by finding the difference between the Kjeldahl-Gunning-Arnold method and the Kjeldahl method modified to include nitrates is unsatisfactory." Other workers have noted this difficulty also. Strowd explained this discrepancy on the possible basis that "appreciable amounts of nitrate were apparently reduced without zinc and salicylic acid." However, no data were presented in support of this conclusion (Strowd, '20). In connection with this phase of the problem at least 3 conditions must be recognized:

(1) The inaccuracy of the official salicylic-thiosulphate method,

occasioned by the presence of water in the sample (Ranker, '25), has made impossible a critical comparison with other methods for the determination of nitrate-nitrogen by difference.

(2) The accuracy of the Kjeldahl-Gunning-Arnold method for the determination of "total nitrogen not including nitrate-nitrogen" only, is generally accepted. In the presence of nitrate-nitrogen, however, this method may not be accurate. Such a possibility must be recognized since nitrates are quite universally present in plants; such forms as Selaginella and celery, for example, have a high nitrate content.

(3) The Devarda method is frequently used as a comparison method to test the accuracy of other methods for the determination of nitrate-nitrogen only, in plant materials. Allen ('15) and Davisson ('18) call attention to the possibility that some organic-nitrogen substances may be acted upon in the process. Such a condition would impair the value of the Devarda method for such comparisons and for unqualified determinations of "nitrate-nitrogen" in plant materials.

The above phases of the problem of nitrogen determination were investigated by making certain analyses, as follows:

1—To determine the accuracy of the Kjeldahl-Gunning-Arnold method for the determination of "total nitrogen not including nitrate-nitrogen" only, in nitrate-free samples to which a known amount of nitrate-nitrogen is added.

2—To determine whether or not nitrate-nitrogen only can be quantitatively determined by finding the difference between the modified official method (Ranker, '25) and the Kjeldahl-Gunning-Arnold method.

3—To continue with the study of the influence of certain details of manipulation upon the accuracy of the modified official method in particular.

MATERIALS

The various determinations and analyses were made of samples taken from the various soil and plant materials listed below. Most of these materials are on the plant side and most of them were in solution when used. At the time of sampling, the solutions were free from any precipitate or suspended matter; if not, they were filtered before samples were taken. All comparable samples were measured into the Kjeldahl flasks (800 cc.) with the same pipette, at the same time and temperature, and all other conditions of making comparable samples were as nearly identical as possible. The numbers assigned to the various materials correspond to the numbers used in the tables and in the text, as indicating the stock materials from which the samples were taken. Though 2 sets of samples may bear the same number they are not necessarily comparable unless they appear in the same table; they may have been measured out at different times and temperatures. The materials analyzed were as follows:

1—*Aspergillus niger.*
2—*Fusarium culmorum.*

Whole cultures, including residual solutions, of the above organisms were cultured under sterile conditions, in 100-cc. flasks containing exactly 25 cc. Pfeffer's nutrient solution to which 1 per cent glucose had been added. The cultures were grown until a vigorous heavy mat had formed. The entire contents of 1 culture flask constituted 1 sample, being transferred without loss to a Kjeldahl flask for determination of nitrogen.

3—*Aspergillus niger.*
4—*Fusarium culmorum.*
5—*Phoma Betae.*

Residual solutions only of the above organisms were cultured, under sterile conditions, in 300-cc. flasks containing 100 cc. of nutrient solution (refer to No. 1 and 2). At time of sampling the cultures were boiled for 5 minutes and filtered. Analyses were made of 25 cc. of the clear filtrate per sample.

6—Tobacco leaves (water extract).
7—Geranium leaves (water extract).
8—Pea leaves and terminals (water extract).
9—Celery leaves and stalks (water extract).
10—Algae mixture, mostly Spirogyra (water extract).
11—Tomato fruits, ripe (water extract).
12—Tobacco leaves and tops (alcohol extract).
13—Geranium leaves (alcohol extract).
14—Pea leaves and terminals (alcohol extract).
15—Celery leaves and stalks (alcohol extract)
16—Tomato fruits, ripe (alcohol extract).

The fresh material of the above plants was ground through a food-chopper; any drippings produced were added; 1 volume of water or 1 volume of 70 per cent alcohol was added; the mixture was boiled (refluxed in the case of alcohol) for 20–30 minutes and filtered hot; 25-cc. samples were taken from the filtrate for analyses.

17—Greenhouse soil plus mushroom compost (water extract).

One kgm. dry material plus 1 L. H_2O; stirred rapidly 4 hrs.; allowe to precipitate; decanted; filtered; 25-cc. samples taken from filtrat

18—Sugar-cane.
19—Sugar-beet.
20—Pea leaves and terminals.

Water extracts of these materials were prepared in the same manner as indicated for Nos. 6–16.

21—Greenhouse soil containing mushroom compost (water extract)
Prepared from same materials and in same manner as No. 17.

22—Mushroom compost only (cold water extract).
Prepared same as No. 17.

23—Mushroom compost (autoclave extraction).
Prepared same as No. 17 except the mixture was autoclaved, not stirred.

24—Mushroom compost, KNO_3.
Twenty-five cc. No. 22 and 74 each, per sample.

25—*Aspergillus niger*, KNO_3.
Twenty-five cc. No. 3 and 74 each, per sample.

26—*Fusarium culmorum*, KNO_3.
Twenty-five cc. No. 4 and 74 each, per sample.

27—Crude peat
(water extract).

28—Sphagnum moss (dry)
(water extract).

29—*Selaginella apus*
(water extract).

30—*Selaginella apus*
(expressed sap).

31—*Selaginella apus*
(alcohol extract).

32—*Selaginella apus*
(water extract).

Prepared same as Nos. 6–16.

Numbers 33–50 refer to various samples of solution cultures of barley, wheat, and peas. These materials were used for qualitative analyses only; the nature and preparation of the samples are indicated in the various tables where the data of their analyses are recorded.

52—Alanin, KNO_3 solution.
Twenty-five cc. alanin solution (1.277 gm. per liter) and No. 74 each, per sample.

53—Asparagin, KNO_3 solution.
Twenty-five cc. asparagin soln. (0.873 gm. per liter) and No. 74 each, per sample.

54—Sugar-cane, KNO_3.
Twenty-five cc. No. 18 and 74 each, per sample.

55—Urea solution.
Two and one-tenths gm. per liter; 25 cc. per sample.

57—Sugar-cane (water extract).
Prepared same as No. 18.

58—Sugar-cane residue, pulp.
Residue from hot-water extractions; washed until free from NO_3 and NH_3.

59—Mushroom compost (water extract).
Prepared same as No. 17.

60—Sugar-beet (water extract).
Prepared same as Nos. 6–16.

61—Sugar-beet residue, pulp.
Residue from hot-water extractions; washed until free from NO_3 and NH_3.

62—Sugar-beet (third water extract).
Third wash water from No. 61, free from NO_3 and NH_3.

64—Urea, KNO_3 solution.
Twenty-five cc. No. 55 and 74 each, per sample.

65—Sugar-beet, KNO_3.
Twenty-five cc. No. 60 and 74 each, per sample.

70—Uric acid solution.
Five gms. per liter; 50 cc. per sample.

71—Taka diastase solution.
Seven and one-half gms. per liter; 50 cc. per sample.

72—Pea seed, germinated.
These seed (Gradus variety) were germinated for 5 days, after which they were mashed in a mortar to a fine pulp in 5 times their weight (dry wt.) of water. This material was free from nitrates; there was a trace of ammonia.

73—Heavy clay-loam soil (water extract).
Prepared same as No. 17.

74—KNO_3 solution.
This solution contained 1.443 gm. of KNO_3 per liter; by analysis, 25 cc. contained $4.79 \pm .005$ mgs. N. In all cases those samples which contained added nitrate-nitrogen received 25 cc. of this solution. The nitrogen content of this solution did not vary throughout the period of these investigations as determined by frequent control analyses of 25-cc. samples.

METHODS

The following methods were used for the determination of nitrogen:

(1) The modified official method as reported by Ranker ('25) for the determination of total nitrogen including nitrate-nitrogen.

(2) A modification of the Devarda method (Ranker, '25) for the determination of nitrate-nitrogen, and for the determination of total nitrogen including nitrate-nitrogen as a comparison method for the modified official method.

(3) The Kjeldahl-Gunning-Arnold method (Association of Official Agricultural Chemists, '25, page 8, No. 24) for the determination of total nitrogen not including nitrate-nitrogen.

The acid and alkali used in titration were standardized against benzoic acid obtained from the U. S. Bureau of Standards (Sample No. 39B). Fiftieth normal acid and alkali were used in all titrations and the normality factors were redetermined frequently to avoid possible errors from this source. All titrations were carried to the complete disappearance of any red tinge as the end-point of methyl red. With N/50 alkali methyl red was found to be much more sensitive than cochineal and the end-point which was used is practically identical with that of cochineal.

Every determination reported in this paper was checked, qualitatively, for the loss of nitrogen at every stage in the process of analysis. The methods of conducting these qualitative control determinations may be grouped under 3 headings:

1. Qualitative tests made during the process of evaporation of the sample under vacuum: All the vapors evolved in this process were passed through a weak solution of sodium bicarbonate and collected in a second flask, both of which are illustrated in fig. 1. The solution thus collected was tested, (A) for the presence of nitrites and nitrates by the diphenylamine test, and (B) for the presence of ammonia by the use of Nessler's reagent.

2. Qualitative tests made following the addition of the salicylic acid mixture to the sample: Any vapors or fumes formed during this process were forced through a weak solution of sodium bicarbonate by blowing gently on the air inlet tube of the device illustrated in fig. 2. In order to obtain as concentrated a solution for the tests as possible, not more than 10–20 cc. of the bicarbonate solution was used as an absorbing solution. The solution thus

obtained was tested, (A) for the presence of nitrites and nitrates by the diphenylamine test, and (B) for the presence of ammonia by the use of Nessler's reagent.

3. Qualitative tests made during the process of acid digestion: The fumes evolved during this process were led through and collected (by the use of a filter pump) in a flask containing approximately 50 cc. of distilled water. The solution thus collected was tested (A) for the presence of nitrites and nitrates by the diphenylamine test and (B) for the presence of ammonia by the use of Nessler's reagent.

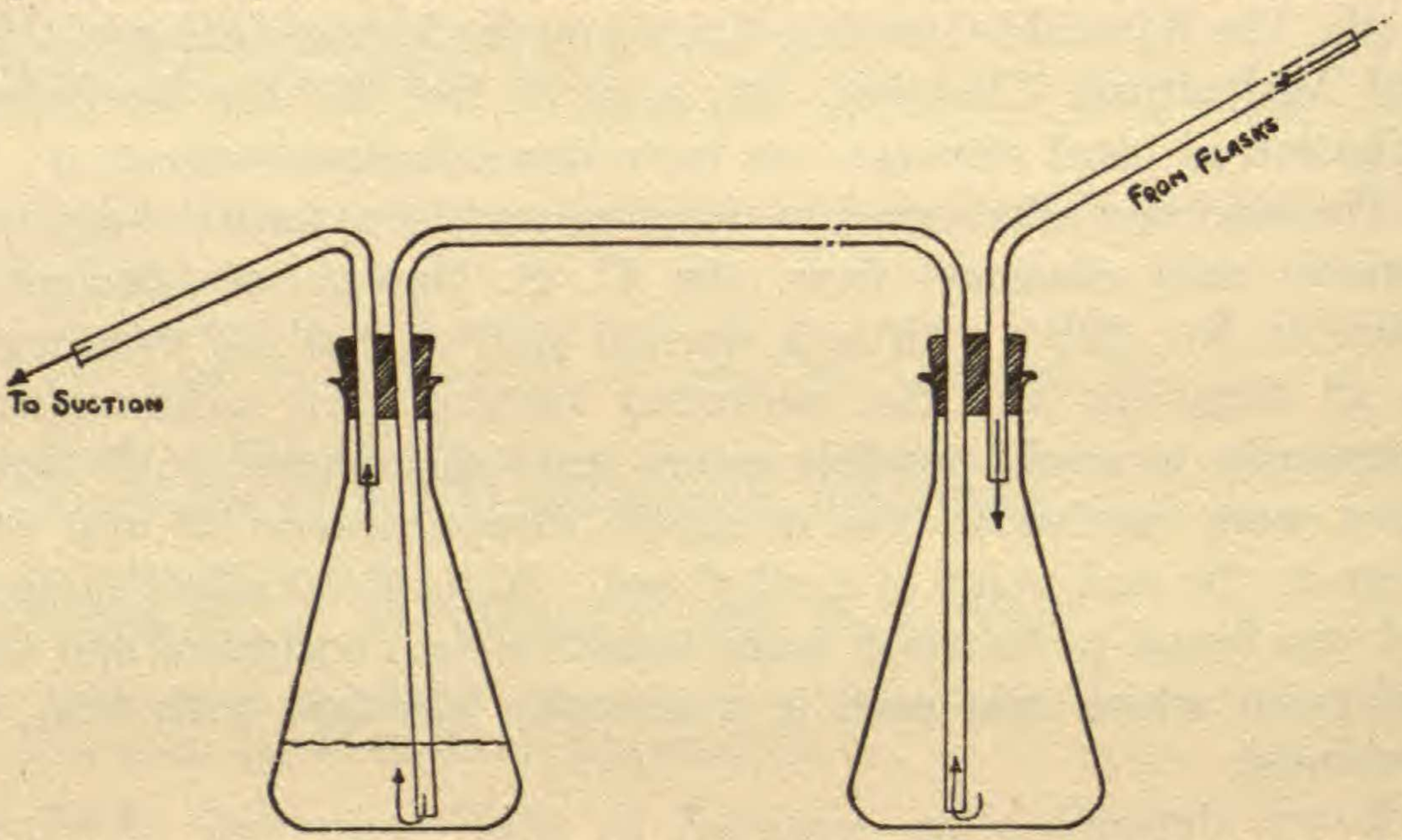

Fig. 1. Arrangement of flasks for the collection of vapors evolved during the evaporation of samples under vacuum and for the collection of fumes evolved during the process of acid digestion.

The 3 groups of qualitative tests, as indicated above, provide a satisfactory check on the accuracy of each stage of the procedure as recommended in the modified official method. Such qualitative tests are a safeguard against carelessness and should be considered as much a part of quantitative procedure as the actual quantitative determination itself. The data obtained from the qualitative tests are presented as the basis for certain recommendations in regard to details of manipulation and procedure. The above capital letters ("A" and "B") are used throughout the various tables as column headings in the same significance as used above; that is, "A" indicates the results obtained with the diphenylamine test and "B" indicates results obtained with Nessler's reagent.

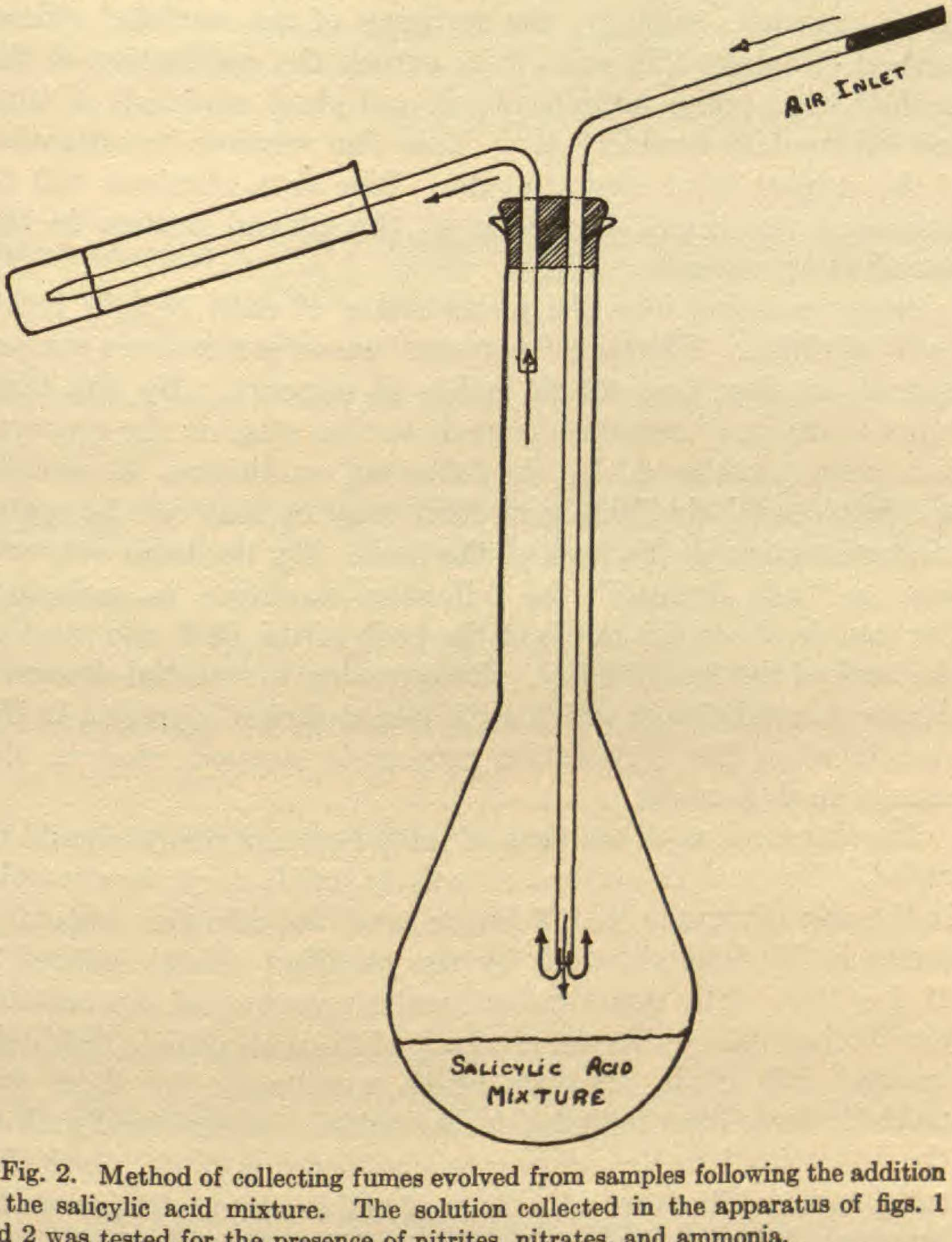

Fig. 2. Method of collecting fumes evolved from samples following the addition of the salicylic acid mixture. The solution collected in the apparatus of figs. 1 and 2 was tested for the presence of nitrites, nitrates, and ammonia.

The evaporation of all samples, which were evaporated, was accomplished on the water bath, under vacuum or as in fig 1.

All probable errors reported in this paper were calculated according to the following formula:

$$\left(E_m = \frac{0.6745\sigma}{\sqrt{n}}\right)$$

Experimental Data

The prime motive in this investigation has been two-fold: (1) to examine, critically, the accuracy of the modified official method (Ranker, '25), and (2) to extend the application of the method to as many samples of soil and plant materials as time and opportunity would permit. This plan required investigation of the subject from several angles. The data obtained will be presented very much according to the general outline in the introductory remarks.

Before entering into the presentation of data certain terms need definition. The term "vacuum" has reference to an equivalent of not less than 22–23 inches of mercury. By the term "just to dryness" reference is made to that stage in the evaporation process evidenced by the following conditions: the sample has just ceased ebullition and there may or may not be water of condensation in the neck of the flask. By the term evaporation to "ash dryness" the following condition is indicated: the sample, the entire inside of the body of the flask and most of the neck of the flask are dry. Evaporation to "partial dryness" denotes a condition in which some free moisture is present in the sample when the evaporation process is stopped; that is, the sample itself is moist.

In connection with the data of table I certain points should be noted. The probable errors are entirely satisfactory; for example, in the case of sample No. 18 (sugar-cane) the nitrogen content as found in 15 determinations by the modified official method is 11.5 ± .006. The quantitative results are checked by suitable qualitative tests so that the accuracy of the procedure is definitely known. All of the analyses which were made by these two methods have been included in the table. In practically all of the determinations the accuracy of the modified official method is satisfactory. There are a few cases of rather serious disagreement, however, between the results obtained by the 2 methods. In this connection the determinations for sample No. 6 (tobacco) are noticed at once. This discrepancy seems to exist, also, with the alcohol extract of this material (No. 12), in each case the determination by the Devarda method being low. After several tests this discrepancy was determined to be due to the volatile

TABLE I

ACCURACY OF THE MODIFIED OFFICIAL METHOD FOR TOTAL NITROGEN IN VARIOUS SAMPLES OF BIOLOGICAL MATERIALS

(arithmetical mean and probable error)

Sample determined		No. of trials	Analysis by Devarda method			Analysis by modified official method				
			Nitrogen found (mgs.)	Qual. tests† for loss of N during digestion		Nitrogen found (mgs.)	Qual. tests†, loss of N			
							During evaporation		During digestion	
No.*	Description*			A‡	B‡		A‡	B‡	A‡	B‡
1	Aspergillus niger (whole cult. incl. soln.)	15	2.2 ± .014	—	—	2.3 ± .009	—	—	—	—
2	Fusarium culmorum (whole cult. incl. soln.)	15	2.3 ± .024	—	—	2.4 ± .010	—	—	—	—
3	Aspergillus niger (residual solution only)	17	0.84 ± .024	—	—	0.83 ± .005	—	—	—	—
4	Fusarium culmorum (residual solution only)	18	1.4 ± .021	—	—	1.4 ± .013	—	—	—	—
5	Phoma Betae (residual soln.)	15	2.1 ± .011	—	—	2.1 ± .030	—	—	—	—
6	Tobacco (water extract)	15	11.4 ± .027	—	—	12.6 ± .039	—	—	—	—
7	Geranium (water extract)	15	3.7 ± .028	—	—	3.9 ± .020	—	—	—	—
8	Garden pea (water extract)	14	21.6 ± .045	—	—	21.8 ± .021	—	—	—	—
9	Celery (water extract)	15	13.1 ± .030	—	—	13.6 ± .009	—	—	—	—
10	Algae mixture (water extract)	15	0.9 ± .003	—	—	0.9 ± .005	—	—	—	—
11	Tomato fruit (water extract)	15	10.0 ± .018	—	—	10.0 ± .021	—	—	—	—
12	Tobacco (alcohol extract)	15	5.3 ± .028	—	—	5.5 ± .007	—	—	—	—
13	Geranium (alcohol extract)	15	6.0 ± .039	—	—	6.2 ± .011	—	—	—	—
14	Garden pea (alcohol extract)	15	9.0 ± .039	—	—	9.7 ± .015	—	—	—	—
15	Celery (alcohol extract)	15	6.1 ± .027	—	—	6.0 ± .018	—	—	—	—
16	Tomato fruit (alc. extract)	15	15.2 ± .009	—	—	15.2 ± .012	—	—	—	—
17	Greenhouse soil containing mushroom compost (water extract)	15	3.9 ± .013	—	—	3.9 ± .006	—	—	—	—
18	Sugar-cane (water extract)	15	11.4 ± .045	—	—	11.5 ± .006	—	—	—	—
19	Sugar-beet (water extract)	15	10.9 ± .067	—	—	10.7 ± .029	—	—	—	—
20	Garden pea (water extract)	15	29.3 ± .053	—	—	29.3 ± .014	—	—	—	—

* The sample numbers correspond to those numbers in the discussion of materials; the method of preparation and description of the samples, also, are given there.

† The methods of conducting these tests are discussed under "Methods."

‡ The letter "A" is used to designate the diphenylamine test for the presence of nitrites and nitrates; "B," to designate the test with Nessler's reagent for the presence of ammonia.

nature of the nicotine contained in the tobacco material. During the alkaline distillation with Devarda's alloy a large part of the nicotine present is volatilized and driven over into the standard acid sample; some of it is absorbed but much of it passes out into the surrounding atmosphere. Such a loss would contribute toward low results by the Devarda method. This condition is demonstrated by the odor of the nicotine given off and may be determined more or less quantitatively by collecting such vapors in sulphuric acid and analyzing after the Kjeldahl method.

Certain other disagreements exist in the data of table I; for example, in samples No. 6, 9, 12–14. In this connection attention must be called to a rather outstanding fact; in all cases (except sample No. 6 as noted above) in which disagreements are evidenced the relatively low magnitude of the probable error obtained by the modified official method indicates its greater accuracy and reliability. This fact is demonstrated by a consideration of the data for sample No. 9 (13.1 ± .03 as compared with 13.6 ± .009), for sample No. 12 (5.3 ± .028 as compared with 5.5 ± .007), and for sample No. 13 (6.0 ± .039 as compared with 6.2 ± .011). An examination of other sets of determinations reveals the same evidence. The disagreement existing in the case of sample No. 8 does not appear in the case of sample No. 20, although both samples were taken from similar materials. Taken as a whole the data indicate certain points worthy of mention: there is rather satisfactory agreement between the methods; many of the determinations are identical; the data are based on a sufficiently large number of determinations; and the superior accuracy of the modified official method is indicated. Satisfactory results were obtained in the analysis of samples high in sugar content; that is, samples having a high reducing power (samples No. 18 and 19 in table I; samples No. 54 and 60 in table II).

The accuracy of the modified official method is shown, further, by the data of table II. The qualitative results given are considered to be of more value as indicators of the accuracy of the method than purely quantitative data only. The possible errors of distillation and titration are eliminated, thus allowing the distinctive processes of the method (evaporation under vacuum, addition of the acid mixture, and digestion) to be checked under more exacting conditions. The procedure followed was exactly that previously recommended (Ranker, '25) except that certain of the samples were not adjusted to neutrality (this phase of the subject will be considered in connection with the data of tables V and VI).

TABLE II

ACCURACY OF THE MODIFIED OFFICIAL METHOD FOR TOTAL NITROGEN AS INDICATED BY QUALITATIVE TESTS ON THE PROCEDURE RECOMMENDED*

Sample analyzed		Qualitative tests made† ||					
		during evaporation		on addition of acid to sample		during digestion	
No.‡	Description‡	A #	B #	A #	B #	A #	B #
21	Greenhouse soil (water extract)	—	—	—	—	—	—
22	Mushroom compost (cold-water extract)	—	—	—	—	—	—
23	Mushroom compost (autoclave extraction)	—	—	—	—	—	—
24	Mushroom compost extract plus KNO_3 solution	—	—	—	—	—	—
25	Aspergillus niger, residual soln. plus KNO_3 soln.	—	—	—	—	—	—
26	Fusarium culmorum, residual soln. plus KNO_3 soln.	—	—	—	—	—	—
27	Crude peat extract (boiled)	—	—	—	—	—	—
28	Sphagnum moss (H_2O extract)	—	—	—	—	—	—
30	Selaginella, expressed sap	—	—	—	—	—	—
31	Selaginella (alcohol extract)	—	—	—	—	—	—
32	Selaginella (water extract)	—	—	—	—	—	—
33	Barley plants (green) plus 10 cc. residual soln. (high in nitrate-N)	—	—	—	—	—	—
34	Same as No. 33 except much lower in nitrate-N	—	—	—	—	—	—
35	Wheat plants (green) plus 10 cc. residual soln. (high in nitrate-N)	—	—	—	—	—	—

TABLE II—Continued

Sample analyzed		Qualitative tests made† ||					
		during evaporation		on addition of acid to sample		during digestion	
No.‡	Description‡	A #	B #	A #	B #	A #	B #
36	Same as No. 35 except much lower in nitrate-N	—	—	—	—	—	—
37	Pea plants (green) plus 10 cc. residual solution. (high in nitrate-N)	—	—	—	—	—	—
38	Same as No. 37 except much lower in nitrate-N	—	—	—	—	—	—
39	Residual soln. from wheat cultures containing 200 mgs. nitrate-N	—	—	—	—	—	—
40	Same as No. 39 except containing 100 mgs. nitrate-N	—	—	—	—	—	—
41	Same as No. 39 except containing 50 mgs. nitrate-N	—	—	—	—	—	—
42	Same as No. 39 except containing 25 mgs. nitrate-N	—	—	—	—	—	—
43	Same as No. 39 except containing 10 mgs. nitrate-N	—	—	—	—	—	—
44	Same as No. 39 except containing 5 mgs. nitrate-N	—	—	—	—	—	—
45 46 47 48 49 50	These samples were the same as those of Nos. 39–44 except that barley plants were used instead of wheat	—	—	—	—	—	—
52	Alanin plus KNO_3 solution	—	—	—	—	—	—
53	Asparagin plus KNO_3 soln.	—	—	—	—	—	—
54	Sugar-cane extract plus KNO_3 solution	—	—	—	—	—	—
60	Sugar-beet (water extract)	—	—	—	—	—	—

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* Ranker ('25).

† The methods of conducting these tests are given under "Methods."

|| The analysis of each separate sample was repeated 5 times; all 5 tests were in agreement, and as indicated for each sample, respectively.

‡ The sample numbers correspond to those numbers used in the discussion of materials.

"A" is used to designate the diphenylamine test; "B," the test with Nessler's reagent.

A critical examination of certain methods for the determination of nitrate-nitrogen has been undertaken in this investigation. This phase of the subject is still under investigation. It seems desirable, however, to include in this report certain results which were obtained. The accuracy of the Kjeldahl-Gunning-Arnold method (Association of Official Agricultural Chemists, '25, page

TABLE III

ACCURACY OF THE KJELDAHL-GUNNING-ARNOLD METHOD[*] FOR THE DETERMINATION OF "TOTAL NITROGEN NOT INCLUDING NITRATE NITROGEN"

(Expressed as cc. N/50 HCl neutralized by the ammonia content of the distillate)

Sample determined		Total N present including nitrate-N	Nitrate-N present[‖]	"Total nitrogen not including nitrate-N"		
No.[†]	Description[†]			Amt. present	Amt. found	% error[‡]
52	Alanin, KNO_3 soln.	29.3 ± .04	17.1 ± .01	12.2 ± .04		
	sample in solution.				13.5	+ 10.7
	sample in solution.				13.0	+ 6.6
	sample dry, evaporated.				12.7	+ 4.1
53	Asparagin, KNO_3 soln.	27.7 ± .06	17.1 ± .01	10.6 ± .06		
	sample in solution				11.9	+ 12.3
	sample in solution.				12.2	+ 15.1
24	Mushroom compost, KNO_3.	25.4 ± .06	17.1 ± .01	8.3 ± .06		
	sample in solution.				9.0	+ 8.4
	sample in solution.				8.7	+ 4.8
	sample dry, evaporated.				10.8	+ 30.1
54	Sugar cane, KNO_3 soln.	49.5 ± .05	17.1 ± .01	32.4 ± .05		
	sample in solution.				38.5	+ 18.8
	sample in solution.				36.8	+ 13.6
	sample dry, evaporated.				41.9	+ 29.3
25	Aspergillus niger, KNO_3.	17.8 ± .04	17.1 ± .01	0.7 ± .04		
	sample in solution.				0.9	+ 28.6
	sample in solution.				1.1	+ 57.1
	sample dry, evaporated.				2.3	+ 228.6
26	Fusarium culmorum, KNO_3.	18.3 ± .05	17.1 ± .01	1.2 ± .05		
	sample in solution.				1.3	+ 8.3
	sample in solution.				2.1	+ 75.0
	sample dry, evaporated.				3.2	+ 166.7

[*] Association of Official Agricultural Chemists ('25), page 8, No. 24.

[†] The sample numbers correspond to those numbers used in the discussion of materials.

[‖] This amount of nitrate-nitrogen was added as 25 cc. of solution No. 74; the other component of the sample was nitrate-free as indicated by a negative diphenylamine test.

[‡] The basis of these calculations was the amount of nitrogen present (column No. 3) in the form of "total nitrogen not including nitrate-nitrogen."

8, No. 24) for the determination of "total nitrogen not including nitrate-nitrogen" is well established. In the analysis of plant materials (in which nitrate-nitrogen is usually present) the accuracy of this method has been assumed rather generally. Its applicability to samples of plant materials has not always been satisfactorily demonstrated. In attempting to investigate the possibility of estimating nitrate-nitrogen, by finding the difference between the modified official method and the Kjeldahl-Gunning-Arnold method, it seemed imperative to determine the accuracy of the latter method when applied to plant materials. The data of table III cover a part of this work.

The outstanding facts obtained from the data of table III are that all the results are high, and that in the presence of nitrate-nitrogen this method is not accurate when applied to plant materials. Other data, not included in this report, support this conclusion and support the suggestion made by Strowd ('20) that "appreciable amounts of nitrate were apparently reduced without zinc and salicylic acid." In all cases the plant extract component of the respective samples was known to be definitely free from nitrates and nitrites, so that the 25 cc. of solution No. 74 (KNO_3) furnished the only nitrate-nitrogen present. In the case of samples Nos. 25 and 26, the experimental error is high due to the low content of organic nitrogen, and the calculation of the "% error" therefore is subject to the same magnitude of error. As a result these data (Nos. 25 and 26) are not entirely satisfactory. It is of interest to note that in all cases, except for sample No. 52, the inaccuracy of the method is greatly increased when the sample is dry. This result is in sharp contrast to the condition of the official salicylic-thiosulphate method (Ranker, '25) in which case low results were obtained unless the sample was dry.

The first attempts to determine nitrate-nitrogen only, by finding the difference between the modified official method and the Kjeldahl-Gunning-Arnold method, were made on samples similar to those included in table III. Later determinations confirmed the earlier results, but only the latter are included in table IV.

The data of table IV support the same conclusions as do those of table III. This condition would be expected from the situa-

TABLE IV

DETERMINATION OF NITRATE-NITROGEN BY FINDING THE DIFFERENCE BETWEEN THE *MODIFIED OFFICIAL METHOD* AND THE *KJELDAHL-GUNNING-ARNOLD METHOD* *

Sample analyzed		Nitrate-N present in sample (mgs.)‖	Total N found by modified official method (mgs.)	N found by K-G-A. method (mgs.)	Nitrate-N found by difference (mgs.) [compare with column I]	% error**
No.	Description					
65	Sugar-beet, KNO_3	4.8 ± .01	15.5 ± .03	11.3	4.2	—12.5†
				11.6	3.9	—18.8†
				11.8	3.7	—22.8‡
54	Sugar-cane, KNO_3	4.8 ± .01	13.9 ± .01	10.8	3.1	—35.4†
				10.3	3.6	—24.9†
				11.7	2.2	—54.2‡
24	Mushroom compost plus KNO_3	4.8 ± .01	7.1 ± .02	2.5	4.6	— 4.2†
				2.4	4.7	— 2.1†
				3.0	4.1	—14.6‡
25	Aspergillus niger plus KNO_3	4.8 ± .01	5.0 ± .01	0.3	4.7	— 2.1†
				0.3	4.7	— 2.1†
				0.6	4.4	— 8.3‡
26	Fusarium culmorum plus KNO_3	4.8 ± .01	5.1 ± .01	0.4	4.7	— 2.1†
				0.6	4.5	— 6.2†
				0.9	4.2	—12.5‡
52	Alanin, KNO_3	4.8 ± .01	8.2 ± .01	3.8	4.4	— 8.3†
				3.6	4.6	— 4.2†
				3.6	4.6	— 4.2‡
53	Asparagin, KNO_3	4.8 ± .01	7.8 ± .02	3.3	4.5	— 6.2†
				3.4	4.4	— 8.3‡

* Association of Official Agricultural Chemists ('25), page 8, No. 24.

‖ This amount of nitrate-nitrogen was added as 25 cc. of solution No. 74; the other component of the sample was nitrate-free as indicated by a negative diphenylamine test.

** The basis of this calculation was the amount of nitrate-nitrogen present in the sample (column I), that is, 4.8 ± .01 mgm. nitrate-nitrogen.

† Sample in solution, that is, just as made up.

‡ Sample first evaporated to dryness on a water bath under vacuum.

tion as noted in the latter case. There seems to be some correlation between the inaccuracy of the method and the presence of certain reducing substances. Certain results which have been obtained, but which are not included in this report, seem to warrant the belief that with certain modifications these difficulties can be overcome and accurate results obtained in the presence of nitrate-nitrogen. With this accomplished it seems very probable that nitrate-nitrogen only can be quantitatively determined by finding the difference between the modified official method and a modification of the Kjeldahl-Gunning-Arnold method.

Early in this investigation it became apparent that certain details of manipulation influenced, more or less, the accuracy of the results obtained. In some cases the procedure followed was determined by these factors. The methods of qualitative controls which were developed (discussed under "Methods") made it possible to determine, accurately, the influence of certain details of procedure. Many of the samples were subjected to extreme conditions in order to determine the limits to which certain procedures could be carried. The data obtained from these various studies are given in tables V–IX.

Certain points in regard to the data of table V should be mentioned. The neutrality of the sample is a very important consideration with most of the samples, Nos. 5 and 5b, 29 and 32, 17 and 21. For such samples, this one factor of neutrality would determine the accuracy of subsequent quantitative analyses. The samples just referred to were on the acid side of neutrality prior to neutralization. On the other hand, when the reaction is alkaline a loss of ammonia may occur (sample No. 26a). Another effect of this factor of neutrality should be noted as evidenced by samples Nos. 5b and 5c, 32 and 32a. Sample No. 5b, *Phoma Betae*, having a P_H value of 6.5, could be quantitatively evaporated just to dryness when neutralized, but when evaporated to ash dryness there was a loss of nitrogen (sample No. 5c). On the other hand, sample No. 32a, having a P_H value of 6.8, could be evaporated to ash dryness without a loss of nitrogen. Of all the materials analyzed throughout this entire investigation *Phoma Betae* was the most difficult. When the factors involved (adjustment to neutrality and evaporation just to dryness) were controlled, however, no difficulty was experienced as is shown by the quantitative results obtained (sample No. 5, table I). Sample No. 25 illustrates the opposite extreme; this sample (*Aspergillus niger*) having a P_H value of 3.9 required no adjustment to neutrality even in the presence of added nitrate-nitrogen. These two materials (Nos. 5 and 25) represent the extremes met with. Somewhat similar variations were observed throughout the entire

TABLE V

INFLUENCE OF NEUTRALITY OF THE SAMPLE UPON THE STABILITY OF NITROGEN DURING THE PROCESS OF EVAPORATION

No.*	Sample analyzed and method of treatment	Qual. tests† for loss of N during evaporation‡	
		A #	B #
5	Phoma Betae residual soln., high in NO_3, P_H 6.5 (*not* adjusted to neutrality, evaporated just to dryness)	+	—
5a	Same as No. 5, except evaporation was less vigorous	+	—
5b	Same as No. 5, except sample *adjusted to neutrality*	—	—
5c	Same as No. 5b, except sample evaporated to ash dryness	+	—
29	Selaginella extract, 14 ds. old. P_H *5.5*, *not neutralized* (evaporated just to dryness)	+	—
32	Selaginella extract, used immediately, P_H *6.8* (evaporated just to dryness)	—	—
32a	Same as No. 32, except sample evaporated to ash dryness	—	—
26	Fusarium culmorum residual soln., plus KNO_3, P_H 8.5	—	—
26a	Same as No. 26, except trace NH_3 added, P_H *8.7*	—	±
17	Greenhouse soil with mushroom compost, H_2O extract, stood in lab. 10 ds., P_H *5.7* (evap. just to dryness)	+	—
17a	Same as No. 17, except extract autoclaved before testing	+	—
21	Sample made from same material as was No. 17, extract analyzed immediately, P_H *7.1*	—	—
25	Aspergillus niger residual soln. plus KNO_3, P_H *3.9* (adjusted to neutrality and evap. just to dryness)	—	—
25a	Same as No. 25, except evaporated to ash dryness	—	—
25b	Same as No. 25, except sample not neutralized (P_H 3.9)	—	—
25c	Same as No. 25a, except sample not neutralized (P_H 3.9)	—	—

* The sample numbers correspond to those numbers used in the discussion of materials.

† The methods of conducting these tests are given under methods.

"A" is used to designate the diphenylamine test; "B," to designate the test with Nessler's reagent.

‡ The analysis of each separate sample was repeated 5 times; all 5 tests were identical and as indicated for each sample, respectively.

list of materials used; some required adjustment to neutrality and others did not.

The method of adjusting a sample to neutrality was found to be of extreme importance to accurate procedure. The influence of this factor is apparent from a consideration of the data of table VI.

TABLE VI

INFLUENCE OF METHOD OF NEUTRALIZING THE SAMPLE UPON THE STABILITY OF NITROGEN DURING THE PROCESS OF EVAPORATION

Sample analyzed and method of treatment*	Nitrogen found in sample (mgs.)	Qual. tests† for loss of N during evaporation	
		A #	B #
(a) Shive's nutrient soln., calculated to contain 200 mgs. nitrogen per 950 cc. (*indicator‡ added to sample*, adjusted to neutrality directly)	184.3	+	—
(b) Same as (a), except *indicator was omitted;* adjusted to neutrality by adding a predetermined amount of alkali	202.7	—	—
(c) Shive's nutrient soln., calculated to contain 100 mgs. nitrogen per 950 cc. (*indicator added to sample*, adjusted to neutrality directly)	96.3	+	—
(d) Same as (c), except *indicator was omitted;* adjusted to neutrality by adding a predetermined amount of alkali	101.3	—	—
(e) Shive's nutrient soln., calculated to contain 50 mgs. nitrogen per 950 cc. (*indicator added to sample*, adjusted to neutrality directly)	47.9	+	—
(f) Same as (e), except *indicator was omitted;* adjusted to neutrality by adding a predetermined amount of alkali	48.4	—	—

* All samples were evaporated just to dryness.

† The methods of conducting these tests are given under "Methods."

# "A" is used to designate the diphenylamine test; "B," the test with Nessler's reagent.

‡ The indicator used for these data was brom-cresol purple.

The data of table VI need little comment; the same qualitative results were obtained with other samples and other indicators. *Phoma Betae* samples were extremely difficult to deal with. The choice of indicators that may be used is limited to those which contain no nitrogen, when the indicator is added directly to the sample.

In the procedure for the modified official method (Ranker, '25, p. 371) it is recommended that the sample be neutralized and then evaporated under vacuum just to dryness. The question of neutrality has been considered in connection with the data of tables v and vi. The study of the factors involved in the process of evaporation was continued, to ascertain the limits of the process and to evaluate the recommendation that the sample be evaporated just to dryness, when applied to various samples. A portion of the results obtained are reported in table vii; the book of data, from which these were taken, contains many more just like those included in table vii. The data included are entirely representative and a sufficient number were taken to show the extremes and the range of variation in the results obtained. In all such analyses the factor of neutrality of the sample was taken care of and controlled; if the sample required neutralization (sample 5, for example) it was adjusted to neutrality by adding a predetermined amount of acid or alkali; if the sample did not require neutralization (samples 33 and 9, for example) it was not neutralized. The data obtained, therefore, may be compared directly in relation to the process of evaporation under vacuum. All of the samples used contained nitrate-nitrogen, either present as shown by a positive diphenylamine test, or as added nitrate-nitrogen, in which case 25 cc. of solution No. 74 was added per sample.

The outstanding fact illustrated by the data of table vii is that the process of evaporation cannot be conducted in any "haphazard" manner. Some samples require rather careful evaporation just to dryness while others may be evaporated to ash dryness and heated for an hour afterward without loss of nitrogen. The most stable material used was No. 3 (*Aspergillus niger*), which with added nitrate-nitrogen (sample No. 25a) could be evaporated to ash dryness with safety. In all cases when the sample was evaporated to partial dryness only, there was a subsequent loss of nitrogen at the time the salicylic acid mixture was added and also during the process of digestion (illustrated by samples No. 33, 35, 37, etc.). The influence of the presence of water in the sample is demonstrated, further, by a consideration of the data for samples Nos. 17 and 17a; these samples were evaporated just to dryness and the acid mixture was added; 5 minutes later a small amount of water was added

TABLE VII

INFLUENCE OF THE EXTENT TO WHICH EVAPORATION IS CARRIED UPON THE STABILITY OF NITROGEN DURING THE PROCESSES OF EVAPORATION, ADDITION OF THE ACID MIXTURE, AND SUBSEQUENT DIGESTION

Sample analyzed*		Qual. tests for loss of nitrogen†					
		during evaporation		on addition of acid to sample		during digestion	
No. ‡	Description‡	A #	B #	A #	B #	A #	B #
33	Barley plants plus 10 cc. residual soln. (evap. to *partial* dryness)	—	—	+	—	+	—
33a	Same as No. 33, except evaporated *just to* dryness	—	—	—	—	—	—
33b	Same as No. 33, except evaporated to *ash* dryness	+	—	—	—	—	—
35	Wheat plants plus 10 cc. residual soln. (evap. to *partial* dryness)	—	—	+	—	+	—
35a	Same as No. 35, except evaporated *just to* dryness	—	—	—	—	—	—
35b	Same as No. 35, except evaporated to *ash* dryness	+	—	—	—	—	—
37	Pea plants plus 10 cc. residual soln. (evap. to *partial* dryness)	—	—	+	—	+	—
37a	Same as No. 37, except evaporated *just to* dryness	—	—	—	—	—	—
37b	Same as No. 37, except evaporated to *ash* dryness	+	—	—	—	—	—
17	Greenhouse soil plus mushroom compost, H_2O extract (evap. just to dryness; acid mixture added; 5 minutes later H_2O added)	—	—	+	—	+	—
17a	Same as No. 17, except H_2O added 24 hrs. after acid mixture	—	—	+	—	+	—
17b	Same as No. 17, except evap. to *ash* dryness, no water added	+	—	—	—	—	—
20	Garden pea, water extract (evap. *just to* dryness)	—	—	—	—	—	—
20a	Same as No. 20, except evaporated to *ash* dryness	+	—	—	—	—	—
5	Phoma Betae residual soln. (evap. *just to* dryness)	—	—	—	—	—	—

TABLE VII—Continued

Sample analyzed*		Qual. tests for loss of nitrogen†					
		during evaporation		on addition of acid to sample		during digestion	
No.‡	Description‡	A #	B #	A #	B #	A #	B #
5a	Same as No. 5, except evaporated to *ash* dryness	+	—	—	—	—	—
5b	Same as No. 5, except evap. to *partial* dryness	—	—	+	—	+	—
26	Fusarium culmorum residual soln. plus KNO_3, evap. *just to* dryness	—	—	—	—	—	—
26a	Same as No. 26, except evaporated to *ash* dryness	—	—	—	—	—	—
26b	Same as No. 26, except evap. to *partial* dryness	—	—	+	—	+	—
25	Aspergillus niger residual soln., plus KNO_3, evap. *just to* dryness	—	—	—	—	—	—
25a	Same as No. 25, except evap. to *ash* dryness	—	—	—	—	—	—
25b	Same as No. 25, except evap. to *partial* dryness	—	—	+	—	+	—
9	Celery, water extract, evap. *just to* dryness	—	—	—	—	—	—
9a	Same as No. 9, except evap. to *ash* dryness	—	—	—	—	—	—
24	Mushroom compost extract plus KNO_3, evap. *just to* dryness	—	—	—	—	—	—
24a	Same as No. 24, except evap. to *ash* dryness	—	—	—	—	—	—

* The analysis of each separate sample was repeated 5 times; all 5 tests were identical and as indicated for each sample, respectively.

† The methods of conducting these tests are given under "Methods."

‡ The sample numbers correspond to those numbers used in the discussion of materials.

"A" is used to designate the diphenylamine test; "B," the test with Nessler's reagent.

and there was a loss of nitrogen; 24 hours later a small amount of water was added to duplicate samples and there was a similar loss of nitrogen. Special attention is called to sample No. 9

(celery). This material was very high in nitrate-nitrogen, second only to sample No. 32 (*Selaginella apus*). Both of these samples required no neutralization and could be evaporated to ash dryness without a loss of nitrogen (since the results were identical the data for sample No. 9 only are reported in this connection).

A special phase of the process of evaporation is illustrated by such materials as whole green plants. When the sample involved contains whole green plants, plus about 10 cc. of the nitrate-containing residual solution, considerable difficulty is experienced in evaporation under vacuum: (1) the colloidal complex of the plant does not give up its water content rapidly in the humid atmosphere inside the flask; (2) the small amount of moisture present in the sample does not evolve sufficient steam to break down effectively the plant structure; (3) the steam evolved under such conditions does not heat the flask sufficiently to prevent condensation and consequent run-back. The semi-equilibrium obtained, however, is such that evaporation may be accomplished in this manner but the time required is too long for efficiency. It required 3–6 hours to evaporate the sample to partial dryness under vacuum, 6–8 hours to evaporate it just to dryness under vacuum, and 7–10 hours, to full dryness under vacuum. These time intervals refer only to the particular kind of sample mentioned above, namely, whole green plants plus about 10 cc. of the nitrate-containing residual solution. Furthermore, it is difficult to judge, under these conditions, when the sample is evaporated just to dryness (sample No. 35, table VIII); if carried on just past this stage there is a loss of nitrogen (sample No. 35a), especially if the sample is high in nitrate-nitrogen. These difficulties may be overcome in one of two ways: (1) by the addition of an appreciable amount (50–100 cc.) of distilled water, sufficient to evolve steam enough to break down completely the plant structure and organization; (2) by the use of some such ventilation device as that illustrated by fig. 3, by which a continuous stream of dry air is used to carry off the vapors from the sample. This latter method is recommended. With this device there was no loss of nitrogen from any sample of whole green plants, even when large amounts of nitrate-nitrogen were added. Some of the data obtained from this study are reported in table VIII, and are representative and typical of all the data obtained.

Certain points in connection with the data of table VIII are worthy of mention. The time periods required to evaporate the samples under vacuum have been given, namely: to evaporate the samples, under vacuum, to partial dryness, 3–6 hours; just to dryness, 6–8 hours; to full dryness, 7–10 hours. In contrast, the time periods required to evaporate similar samples to comparable stages of dryness, by using the special ventilation device (fig. 3), were 10–15 minutes, 12–20 minutes, and 15–25 minutes, respectively. When this latter method was used to evaporate such samples there was no loss of nitrogen in any of the tests made. Sample No. 35c, for example, was heated for 1 hour after it was completely dry, with no loss of nitrogen.

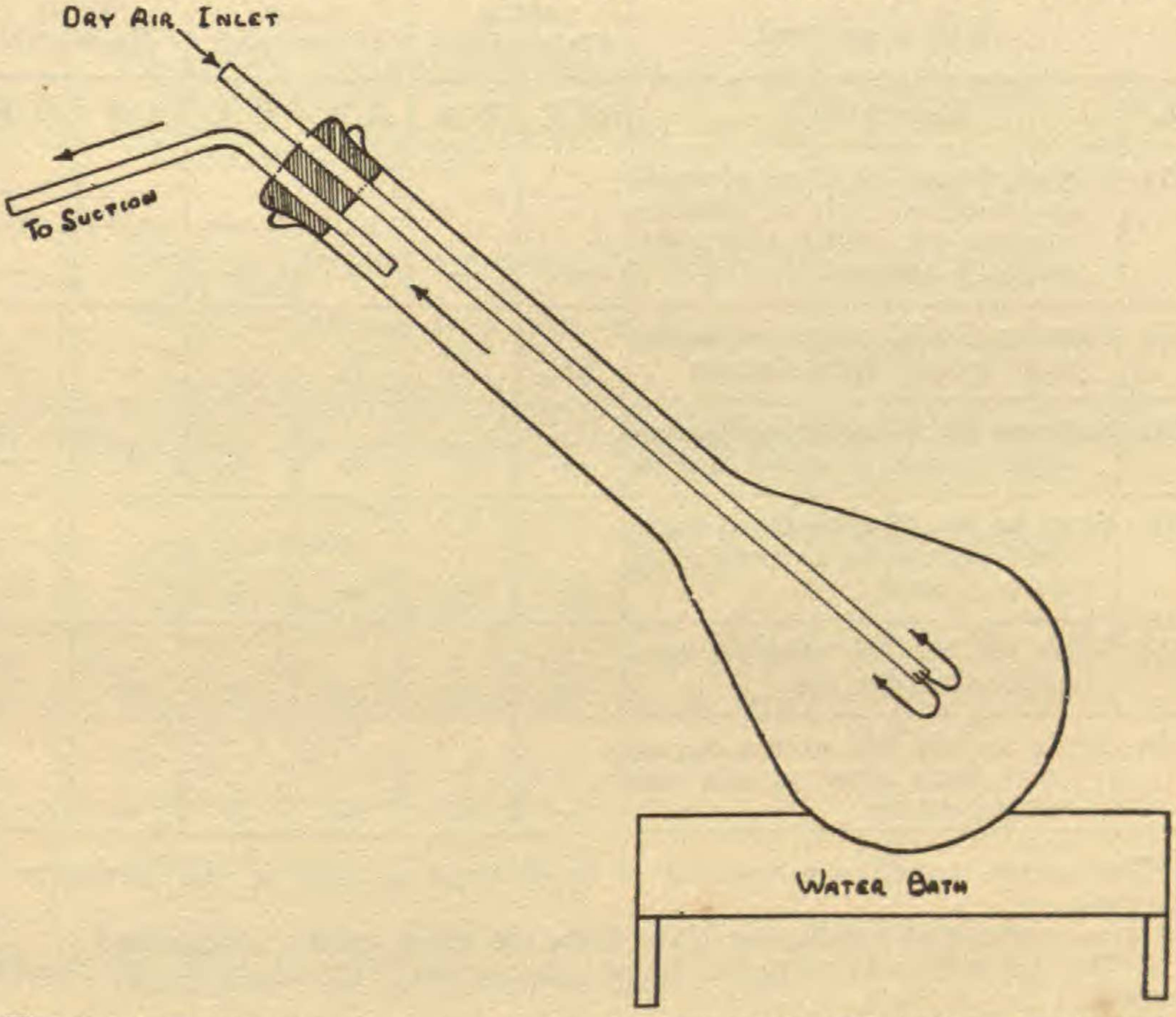


Fig. 3. Arrangement of apparatus for the evaporation of whole green plants by dry-air ventilation.

Very early in this investigation it was noticed that certain irregular results in titration were obtained. For example, in the

TABLE VIII

INFLUENCE OF THE METHOD OF DRYING WHOLE GREEN PLANTS UPON THE STABILITY OF NITROGEN DURING THE PROCESSES OF EVAPORATION, ADDITION OF THE ACID MIXTURE, AND SUBSEQUENT DIGESTION

Sample analyzed		Qual. tests for loss of nitrogen†					
		during evaporation‡		on addition of acid to sample†		during digestion‡	
No.*	Description*	A #	B #	A #	B #	A #	B #
35	Wheat plants plus 10 cc. of residual solution high in nitrate-nitrogen *evaporated under vacuum just to dryness*	—	—	—	—	—	—
35a	Same as No. 35 except *evaporated under vacuum to full dryness*	+	—	—	—	—	—
35b	Same as No. 35 except *evaporated under vacuum to partial dryness*	—	—	+	—	+	—
35c	Same as No. 35 except *evaporated just to dryness by a special ventilation device***	—	—	—	—	—	—
35d	Same as No. 35c except evaporated to full dryness	—	—	—	—	—	—
35e	Same as No. 35c except heated for 1 hour after sample was completely dry	—	—	—	—	—	—

*The sample numbers correspond to those numbers used in the discussion of materials.

† The methods of conducting these tests are given under "Methods."

"A" is used to designate the diphenylamine test; "B," the test with Nessler's reagent.

‡ The same results were obtained for barley and pea plants, samples No. 33, 34, 37, 38, and for wheat sample No. 36. Each test was repeated 5 times for each separate sample and the same results were obtained each time.

** See fig. 3.

simple determination of nitrogen present in a known solution of ammonium sulphate, 2 each of the 6 titrations consistently required more alkali to titrate the excess acid to neutrality than did each of the other 4; also, these same 2 flasks contained a more or less milky distillate that was quite distinctive from the crystal-clear distillate of the others. After several attempts to locate the cause of this difficulty it was noticed that whenever a certain grade of rubber tubing was used, to connect the condenser bulb with the block tin condenser tube of the distillation apparatus,

TABLE IX

INFLUENCE OF RUBBER TUBING CONNECTIONS* UPON THE QUANTITATIVE DISTILLATION OF AMMONIA INTO STANDARD ACID

Sample analyzed and nature of rubber tubing connections used		Cc. N/50 acid neutralized by ammonia contained in distillate	Error (cc. N/50 acid)
No.†	Description		
11	Tomato fruits, water extract		
	Distillation through connection No. 2; average of 4 tests‖	39.1 ± .08	
	Distillation through connection No. 1‡ used on condenser bulb No. 3	37.6	—1.5
9	Celery, water extract		
	Distillation through connection No. 2; average of 3 tests‖	51.9 ± .04	
	Distillation through connection No. 1‡		
	used on condenser bulb No. 3	50.9	—1.0
	used on condenser bulb No. 4	50.0	—1.9
17	Greenhouse soil and mushroom compost, H_2O extract		
	Distillation through connection No. 2; average of 4 tests‖	17.2 ± .02	
	Distillation through connection No. 3 # used on condenser bulb No. 5	12.7	—4.5
73	Heavy clay loam soil, water extract		
	Distillation through connection No. 2, average of 4 tests‖	3.2 ± .03	
	Distillation through connection No. 1‡		
	used on condenser bulb No. 1	2.6	—0.6
	used on condenser bulb No. 2	2.9	—0.3
	used on condenser bulb No. 3	2.9	—0.3
	used on condenser bulb No. 4	2.6	—0.6
18	Sugar-cane, water extract		
	Distillation through connection No. 2; average of 5 tests‖	44.7 ± .06	
	Distillation through connection No. 1‡		
	used on condenser bulb No. 1	43.9	—0.8
	used on condenser bulb No. 2	43.1	—1.6
	used on condenser bulb No. 3	42.9	—1.8
	used on condenser bulb No. 4	40.9	—3.8
	used on condenser bulb No. 5	44.4	—0.3

* As used in this table, the term "connection" refers only to that length of rubber tubing used to connect a glass condenser bulb to a block tin condenser tube of the Kjeldahl distillation apparatus.

† The sample numbers correspond to those numbers used in the "Discussion of materials."

‡ Connections No. 1 were such that about 6 cm. of the total length was exposed to ammonia vapors during distillation. Before use the rubber was extracted once with N/10 NaOH, washed in dilute HCl, and rinsed thoroughly. The rubber tubing used was the ordinary white laboratory grade, thick-walled, cloth-wrapped (A. H. Thomas' No. 8830).

‖ Connections No. 2 were such that not more than 0.5 cm. of the total length was exposed to ammonia vapors during distillation. Before use the rubber was treated as in the case of connections No. 1. The rubber tubing used was A. T. Thomas' No. 8834: black gum and sulphur; steam-cured; thick-walled; smooth bore; of fairly good quality. These connections were adopted for all subsequent work.

Connections No. 3 were the same as connections No. 1 except that about 20 cm. of the total length was exposed to ammonia vapors during distillation.

these milky distillates were obtained. In such distillates the calculated nitrogen content was invariably low. The particular grade of rubber tubing which gave these results was that described in the footnote to table IX and was used in making the "connections No. 1" as indicated. When extracted with N/10 sodium hydroxide a yellow-green sodium sulphide mixture was obtained even after 6 such extractions. This grade of rubber is entirely unsatisfactory for such connections. A better grade of rubber tubing was obtained; this is described in the footnote to table IX and was used to make "connections No. 2" as indicated. (Note that the total length of rubber exposed to the ammonia fumes was not more than .5 cm.). The first extraction of this rubber with N/10 sodium hydroxide produced the characteristic yellow-green sodium sulphide mixture but all subsequent extractions were free from this material. Six check distillations of ammonia, through connections No. 2, produced a probable error of + .0045; these results were entirely satisfactory and this grade of rubber tubing was adopted for all subsequent work. Certain few analyses were made to illustrate the influence of these different rubber connections and these data are given in table IX.

GENERAL DISCUSSION AND CONCLUSIONS

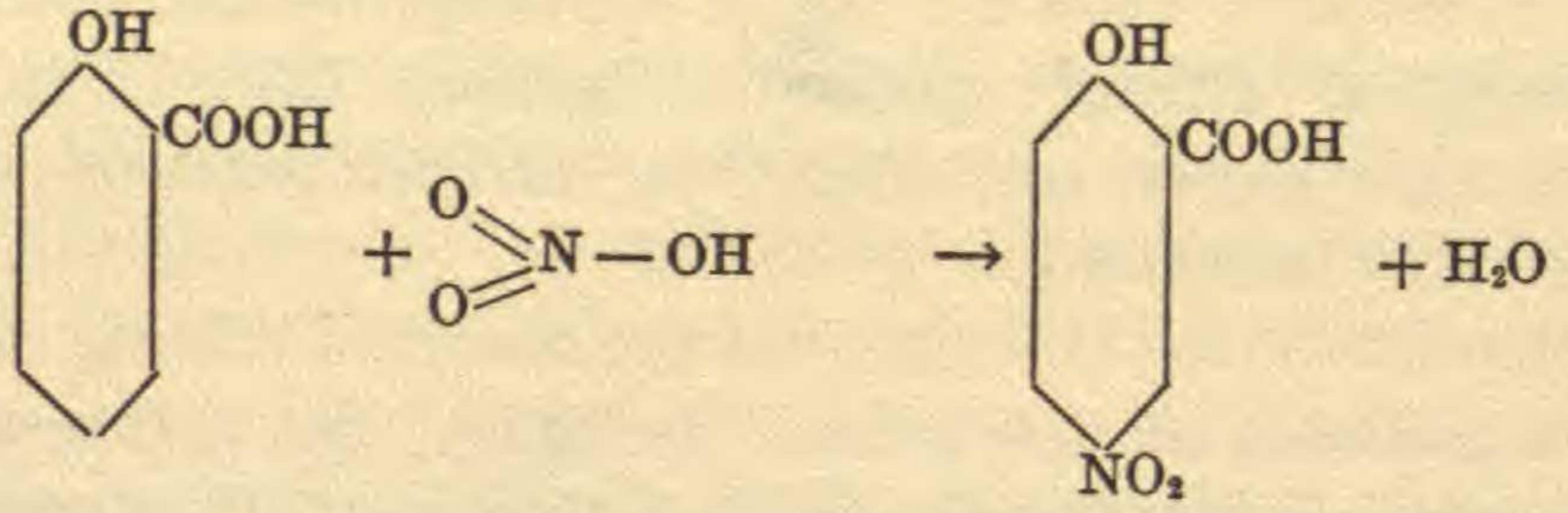
There are certain aspects of the data presented which require some discussion and which require correlation with the data previously reported (Ranker, '25) and with the larger aspects of the determination of nitrogen. In the determination of any form of nitrogen it would seem logical, first, to obtain a method which is accurate for the determination of total nitrogen. Two methods are used generally: (1) some modification of the Devarda method, and (2) the official salicylic-thiosulphate method (Association of Official Agricultural Chemists, '25, p. 9, No. 29). The first method is time-consuming; there is a preliminary distillation with Devarda's alloy, an acid digestion, and a second alkaline distillation. The second method is not accurate when applied to most plant materials in the presence of water (Ranker, '25). This method, however, is relatively rapid when compared with the Devarda method. It is considered that the inaccuracies of this second method (official salicylic-thiosulphate method) have been overcome, with no loss of rapidity, by the procedure for the modified official method (Ranker, '25). The data reported in this paper are offered in further support of the accuracy of this method.

Certain aspects of the chemistry of the modified official method should be considered at this point. Roughly, the nitrogen content of all materials may be divided into 2 classes: (1) organic, and (2) inorganic. Either of these may have an acid or basic reaction. The data of this investigation show that the acid or basic nature of the various forms of nitrogen, as such, does not seem to influence the stability of the total nitrogen contained in the sample. On the other hand, the reaction of the sample seems to determine the stability of its nitrogen-content in many cases. Both acid and basic forms of nitrogen may be present in rather equal proportions in the same sample. Such an occurrence may explain why it is necessary to adjust certain samples to neutrality prior to evaporation. Such an adjustment to neutrality may exert a stabilizing influence, due directly to the relative decrease of hydrogen- or hydroxyl-ions. It may exert, also, an indirect influence, due to the formation of new nitrogen compounds which are more stable than those forms of nitrogen present prior to adjustment of the sample to neutrality.

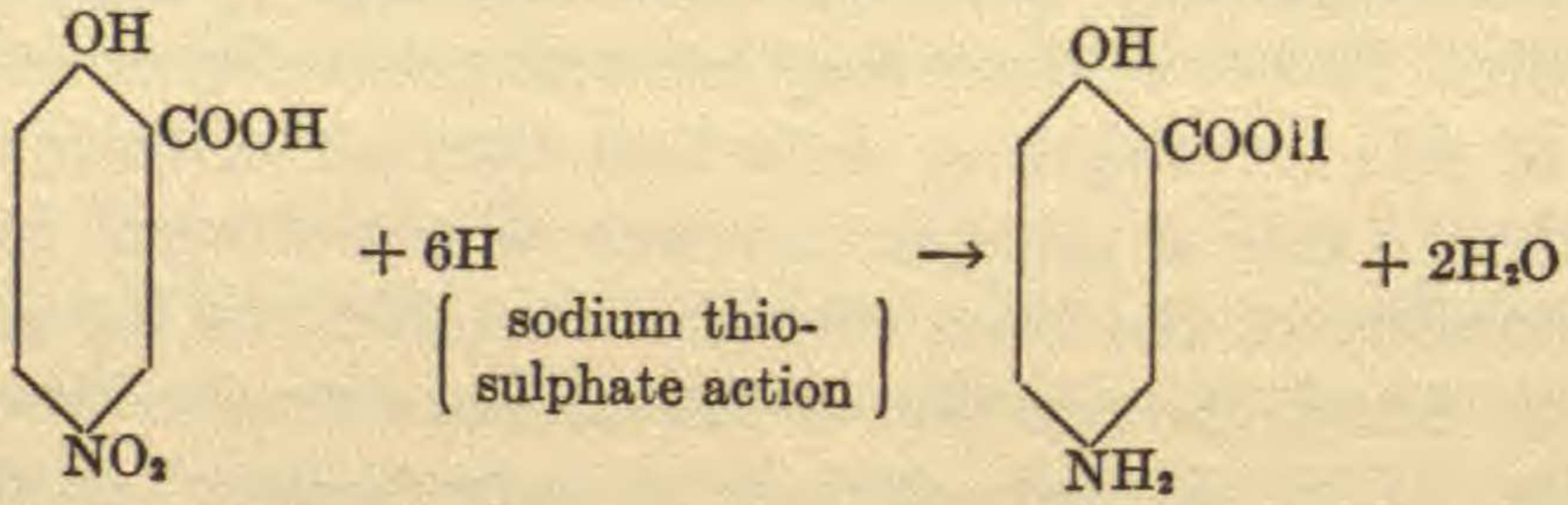
In regard to the organic nitrogen which is present, it would be transformed into ammonia-nitrogen very much as would occur with the Kjeldahl-Gunning-Arnold method and need be considered no further here. The inorganic nitrogen content of a sample, however, presents a different situation. Of the various forms of inorganic nitrogen, nitrate-nitrogen commands most attention. Given the proper condition of dryness (evaporation just to dryness) for a nitrate-containing sample, the reduction of nitrate-nitrogen to ammonia-nitrogen may be considered to take place somewhat as follows, with the use of salicylic acid:

(1) $NaNO_3 + H_2SO_4 \rightarrow NaHSO_4 + HNO_3$

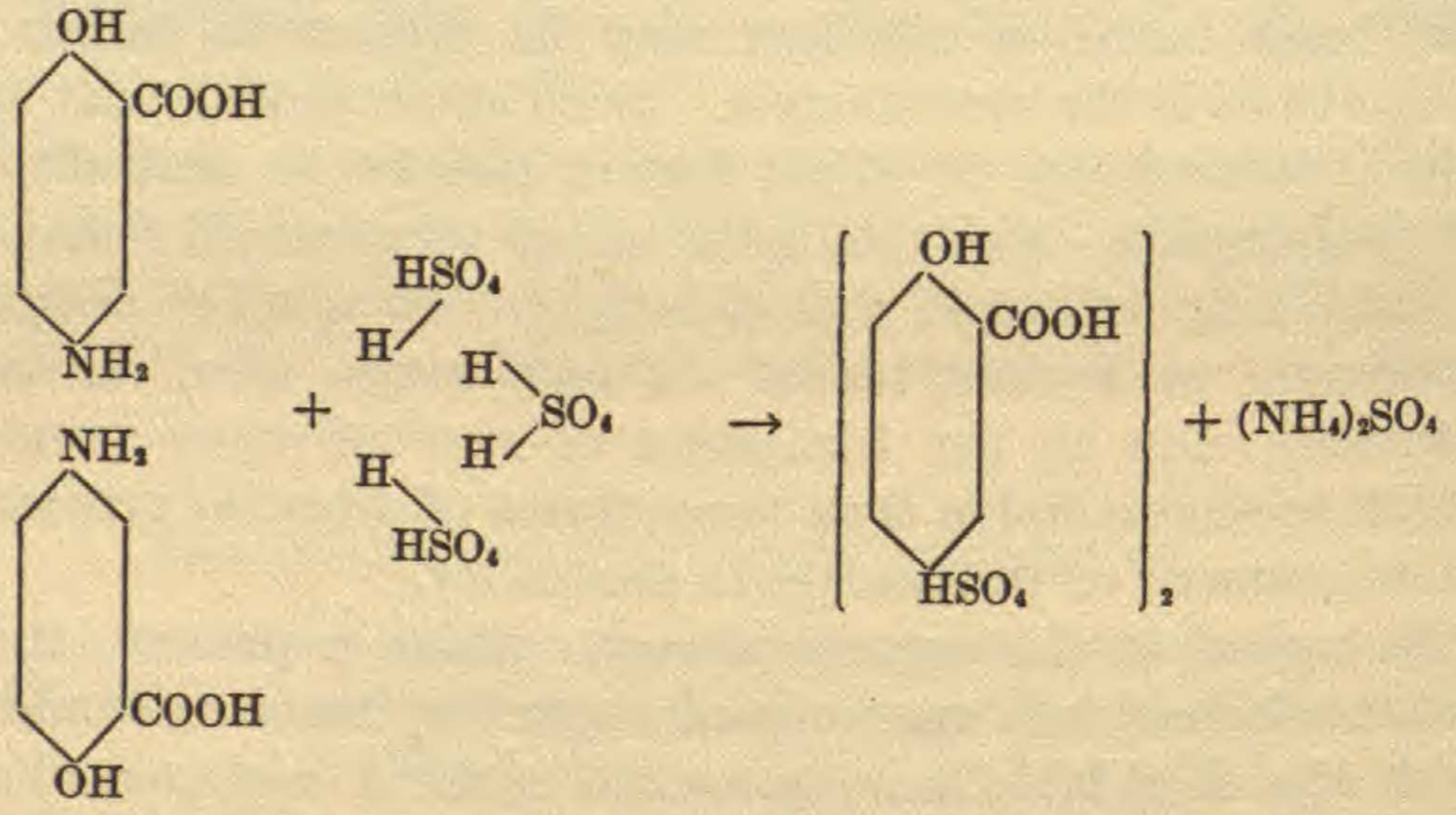
(2)


OH
COOH
+
O
N — OH
O
→
OH
COOH
NO₂
+ H₂O

(3)


OH
COOH
NO₂
+ 6H
sodium thio-
sulphate action
→
OH
COOH
NH₂
+ 2H₂O

(4)


OH
COOH
NH₂
NH₂
COOH
OH
+
HSO₄
H
H
SO₄
H
H
HSO₄
→
OH
COOH
HSO₄
2
+ (NH₄)₂SO₄

By equation (2) it is evident that nitric acid is responsible for the nitrification of the salicylic-acid molecule. The nitric acid is obtained from the nitrates present in the sample (equation 1) by the action of the sulphuric acid which is present in the salicylic-acid mixture (1.0 gm. salicylic acid to 30 cc. sulphuric acid). The sulphuric acid plays another important rôle. The quantitative completion of equation (2) and equation (3) toward the right depends upon the loss of water. For each atom of nitrogen thus combined 3 molecules of water are formed. This water is removed from the reaction by the desiccating action of the sulphuric acid. The reactions (equations 2 and 3), thereby continue to quantitative completion and there is no loss of nitrogen, providing the sample was first evaporated to dryness (Ranker, '25). The sulphuric acid functions in yet another manner, namely, in the digestion of the amino-salicylic-acid molecule and the formation of ammonium sulphate (equation 4).

The various steps in the procedure recommended for the modified official method have been reinvestigated and the results obtained do not warrant any alterations in the procedure. Certain qualifying conditions must be recognized, however: (1) some samples must be adjusted to neutrality prior to evaporation while other samples require no such adjustment; (2) some samples must be carefully evaporated just to dryness and other samples may be roughly evaporated to ash dryness without a loss of nitrogen (the particular chemical or physical complexes responsible for these differences are not known); (3) in the distillation process paraffin may be omitted in some cases but must be present in others to prevent foaming. In just which cases it is safe to take advantage of these qualifying conditions, in order to simplify any step in the procedure, must be decided by the individual investigator using the method. Inasmuch as these factors (neutralization and evaporation just to dryness) are essential to the accurate determination of total nitrogen in some samples they cannot be omitted from the general procedure recommended (Ranker, '25, p. 371).

The above considerations lead directly to a consideration of the importance of the qualitative tests. Without some such tests it is impossible to control, accurately, the various factors involved in the quantitative determination of nitrogen by any of the acid digestion methods, impossible to know, definitely, that there has or has not been a loss of nitrogen in the process, and very difficult to locate any source of error that might exist. It is strongly recommended that suitable qualitative tests (those used in this investigation have been found satisfactory) be considered as an integral part of nitrogen-determination methods whenever it is at all feasible to use them. An example or two might be of value to illustrate this suggestion: (1) certain of the factors contributing to the inaccuracies of methods investigated by Mitscherlich and Herz ('09) could have been definitely located and corrected in this manner; (2) had such tests been used Strowd ('20), using samples containing plant materials plus nitrate-nitrogen in solution, probably would have detected the inaccuracy of the "Kjeldahl method modified to include nitrate" under such conditions; (3) the use of such qualitative tests would have deterred Gallagher ('23, p. 67) from his theoretical denunciation of the principle of reduction of nitrates in acid medium; (4) the data presented in this report are filled with instances in which all sorts of results would have been obtained had not the quantitative data been checked with qualitative tests on the procedure used. Other examples of a similar nature are abundant. It is fortunate, indeed, that the various methods for the determination of nitrogen are so well adapted to the use of qualitative control tests for the loss of nitrogen. It is unfortunate that these qualitative tests are so seldom used.

One other aspect of this investigation should be mentioned at this time, namely, the determination of nitrate-nitrogen. Consideration of this subject will be restricted to the determination of nitrate-nitrogen by finding the difference between the Kjeldahl-Gunning-Arnold method and the modified official method (a separate report is being prepared on certain phases of the determination of nitrate-nitrogen in plants by the Devarda method). The inaccuracies of the official salicylic-thiosulphate method (Association of Official Agricultural Chemists, '25, p. 9, No. 29) in the presence of moisture have prevented the determination of nitrate-nitrogen by finding the difference between that method and the Kjeldahl-Gunning-Arnold method. With the more accurate procedure of the modified official method such a determination of nitrate-nitrogen was thought possible. The inaccuracies of the Kjeldahl-Gunning-Arnold method when applied to samples containing nitrates (data of table III), however, prevent this possibility. The data presented in this report include only negative results in this direction. Certain tentative modifications which have been tried, however, indicate the possibility of perfecting the Kjeldahl-Gunning-Arnold method to the point that "total nitrogen not including nitrate-nitrogen" only, can be quantitatively determined in the presence of nitrate-nitrogen. With this accomplished it is very probable that nitrate-nitrogen only can be quantitatively determined by finding the difference between the modified official method and a certain modification of the Kjeldahl-Gunning-Arnold method.

The various data which have been obtained in this investigation seem to warrant the following conclusions:

1. The modified official method (Ranker, '25) is accurate for the determination of total nitrogen in soil extracts and in samples of biological materials, on the plant side.

2. The Kjeldahl-Gunning-Arnold method (Association of Official Agricultural Chemists, '25, p. 8, No. 24) for the determination of "total nitrogen not including nitrate nitrogen" is not accurate in the presence of nitrate-nitrogen. Until this method is perfected, so that it is accurate in the presence of nitrate-nitrogen, it is useless to attempt the determination of nitrate-nitrogen only, by finding the difference between the modified official method and the present Kjeldahl-Gunning-Arnold method.

3. The data obtained do not warrant any alteration in the recommended procedure for the modified official method (Ranker, '25). The stability of the nitrogen complex of certain samples may permit of certain deviations from the recommended procedure, however. This is a matter for individual judgment and will be governed by the particular samples under investigation. These deviations can be made safely, only when suitable qualitative tests are used to detect a loss of nitrogen from the sample.

4. Suitable, accurate qualitative tests for the loss of nitrogen should be as much an integral part of the various quantitative nitrogen methods as the actual quantitative determination itself, whenever it is possible to use them without decreasing the accuracy of the quantitative method involved.

SUMMARY

1. The accuracy of the modified official method for the determination of total nitrogen, as previously reported, is verified.

2. The application of the modified official method is extended to a wide variety of samples, including soil extracts and samples of biological materials, on the plant side.

3. It is demonstrated that the Kjeldahl-Gunning-Arnold method, for the determination of "total nitrogen not including

nitrate-nitrogen," is not accurate in the presence of nitrate-nitrogen.

4. Until the Kjeldahl-Gunning-Arnold method is modified to be accurate in the presence of nitrate-nitrogen, it is useless to attempt the determination of nitrate-nitrogen by finding the difference between this method and the modified official method.

5. Certain details of manipulation and procedure are discussed, and their influence on the accuracy of methods for nitrogen determination is demonstrated.

6. The value of suitable qualitative tests, for loss of nitrogen, to be used as an integral part of quantitative methods for the determination of nitrogen, is demonstrated, discussed, and the use of such tests is recommended.

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Literature Cited

Allen, E. R. ('15). The determination of nitric nitrogen in soils. Jour. Ind. and Eng. Chem. **7**: 521–529. 1915.

Association of Official Agricultural Chemists ('25). Official and tentative methods of analysis. Washington, 1925.

Davisson, B. S. ('18). Ammonia and nitric nitrogen determinations in soil extracts and physiological solutions. Jour. Ind. and Eng. Chem. **10**: 600–605. 1918.

Gallagher, P. H. ('23). Estimation of nitric-nitrogen and total-nitrogen in plant tissue extracts. Jour. Agr. Sci. **13**: 63–68. 1923.

Mitscherlich, E. A., und Herz, P. ('09). Eine quantitative Stickstoffanalyse für sehr geringe Mengen. Landw. Jahrb. **38**: 279–318. 1909.

Ranker, E. R. ('25). Determination of total nitrogen in plants and plant solutions: a comparison of methods with modifications. Ann. Mo. Bot. Gard. **12**: 367–380. 1925.

Strowd, W. H. ('20). The determination of nitrites and nitrates in plant tissue. Soil Sci. **10**: 333–342. 1920.